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PERSISTENT ELEVATION OF NOCTURNAL ACTIVITY IN RODENTS FOLLOWING APPARENT RECOVERY FROM LITHIUM/PILOCARPINE-INDUCED LIMBIC SEIZURES[1]

M. A. PERSINGER AND S. A. KOREN

Behavioral Neuroscience Laboratory
Laurentian University

Summary.—The nocturnal and day-time activity of rats was monitored continuously after the induction of seizures by a single systemic injection of lithium followed 4 hr. later by the muscarinic agent pilocarpine. Although there was a transient increase in activity during the day cycle shortly after the seizure induction, this behavior returned to baseline levels; however, nocturnal activity increased and remained elevated two to three times above the baseline levels. Implications for the enhancement of nocturnal melatonin levels within the brains of these chronically epileptic rats are discussed.

Rats in which seizures have been induced by a single, systemic injection of lithium (3 mEq/kg) and pilocarpine (30 mg/kg) become chronically epileptic as inferred by the occurrence of episodes of rearing, forelimb clonus, and falling (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993). Although their general activity during the day appears qualitatively normal after the recovery from a lateral hypothalamic-like syndrome during the 5 to 10 days subsequent to the induction of the seizures, these rats display marked elevations in the amount of intermale aggression and hypersexuality during the night (Persinger, 1994). We hypothesized that a relative increase in nocturnal ambulatory activity, relative to baseline, should also be evident in rats in which these types of seizures have been induced.

METHOD

Subjects

During three separate blocks of experiments a total of 12 male Wistar rats, 90 to 100 days old (4 rats per block), were housed singly in one of four plastic 32-cm by 36-cm by 45-cm cages. The rats had been obtained from Charles River (Quebec).

Procedure

Food and water were available *ad libitum*. Corncob bedding was changed once per week during which time the rats were disturbed for not

[1]Please send correspondence and reprint requests to Dr. M. A. Persinger, Behavioral Neuroscience Laboratory, Laurentian University, Ramsey Lake Road, Sudbury, Ontario P3E 2C6 Canada or e-mail (Mpersinger@admin.laurentian.ca).

more than 5 min. The cages were housed within a temperature controlled room (20° to 21° C). The light:dark cycle was 12:12, with local night beginning at 1930 hours local time.

Activity was monitored by the total numbers of times an infrared photoelectric sensor unit (Radio Shack Model 49-551) was interrupted per 10-min. interval. The beam of the photocell traversing the long axis of the clear plastic cage was situated between 7 cm and 10 cm above the cage's floor. The numbers of interruptions were monitored continuously and were recorded by a Commodore C64 computer. Evaluation of successive 10-min. increments over a total of 10 randomly selected 24-hr. periods did not reveal any bin with excessive numbers that would indicate artifactual inflation of the counts from higher frequency microactivity (grooming).

After approximately 15 days, when each rat's individual nocturnal and day-time activity exhibited less than 5% variability for five successive days, each rat was injected subcutaneously with 3 mEq/kg of lithium chloride and then 4 hr. later (in another room) with 30 mg/kg of pilocarpine. Within 30 min. to 40 min. after the display of the overt seizure (rearing, rapid forelimb clonus and falling), 25 mg/kg of acepromazine (Atravet) was injected subcutaneously to facilitate survival. The rats were then returned immediately to their home cage environment.

The mean numbers of disruptions of the photobeam per 10 min. during the 12 hr. of light and the 12 hr. of darkness were recorded by computer during the subsequent 25 days. These values were then divided by the mean numbers of disruptions of the photobeams during the day-time or night-time for the baseline period for each rat separately. The ratios were employed as the indices of change in ambulatory activity. For ease of comparison, the ratios were aggregated into blocks of five successive days (five blocks). Repeated-measure analyses of variance were completed separately for the ratios of activity for the average of the five baseline days (pretreatment block) and the five subsequent blocks after the induction of the seizure. *Post hoc* analyses involved t tests for correlated data. In all analyses SPSS software on a VAX 4000 computer were used.

Results

The means for the ratios of all eight rats' individual activity after the induction of the seizures relative to the baseline levels during the day and night cycle are shown in Fig. 1. Repeated measures analysis indicated a statistically significant ($F_{5,35}=6.10$, $p<.001$; $\eta^2=46\%$) change in day-time activity during the experiment. *Post hoc* correlated t tests indicated that the major source of this effect was due to the transient, relative increases in day-time activity during the blocks that included one to five days ($t=2.48$, $p<.05$) and six to ten days ($t=3.07<.05$) after the seizure induction. After this

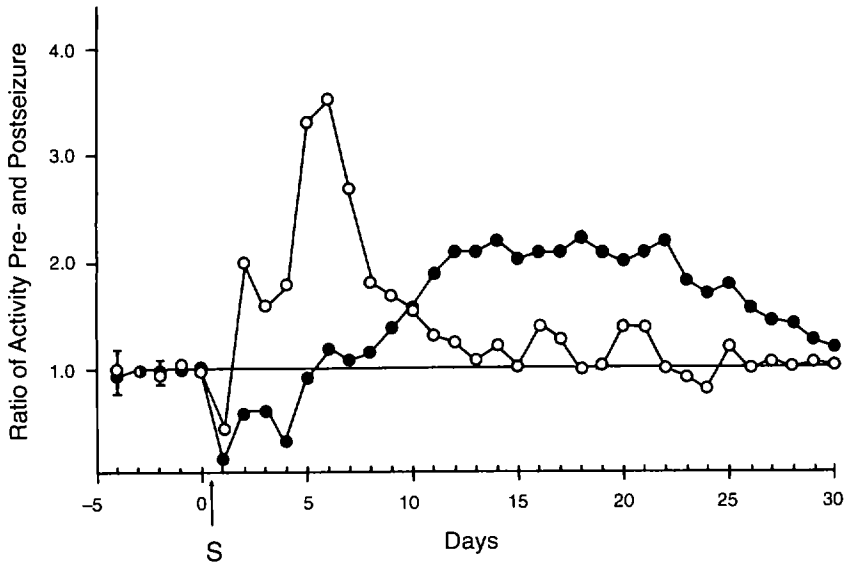

FIG. 1. Means of individual ratios of activity to baseline values (–5 to 0 days) for eight rats after seizure induction (S) during the subsequent 25 days during the daytime and nighttime periods: (o) light (0700–1900) and (•) dark (1901–0659)

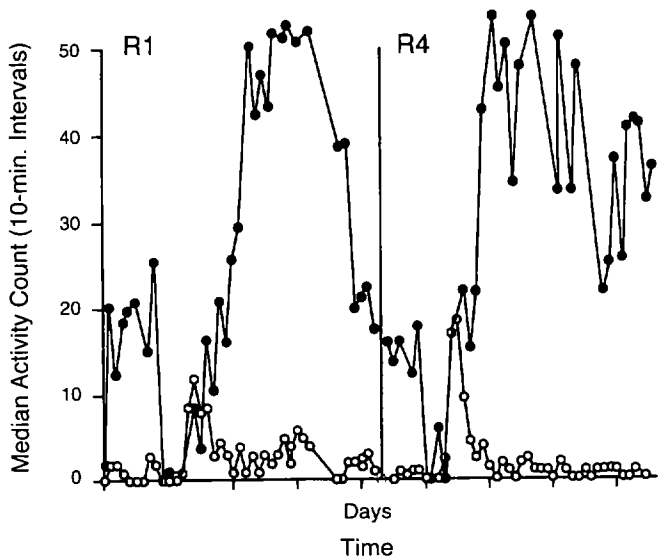

FIG. 2. Means numbers of (raw) activity counts by photocell during the baseline and after seizure induction during the light and dark cycles for two sample rats (R1, R4): (o) day and (•) night

period the differences between the ratios of daytime activity were no longer significantly different from baseline.

Repeated-measures analyses of variance gave a statistically significant difference ($F_{5,35}=21.21$, $p<.001$; $\eta^2=75\%$) between the blocks of five consecutive, 12-hr. nocturnal periods during the 30 days of observation (one block baseline; five blocks after the seizure induction). *Post hoc t* tests indicated that the major source of this difference was due to the elevated nocturnal activity that was evident 10 to 15 days ($t=6.73$), 16 to 20 days ($t=4.87$), and 21 to 25 days ($t=4.11$) after induction of the seizures (after the rats' day-time activity recovered and their behaviors appeared normal) relative to baseline levels. Examples of the mean numbers of counts per 10-min. bin for the light and dark periods for two sample rats from this experiment are also presented (Fig. 2) for examination.

Discussion

The hypothesis that rats in which limbic seizures and brain damage had been induced by a single systemic injection of lithium and pilocarpine would display chronic elevated ambulatory activity during the night phase was supported by the results of this study. The transient occurrence of relatively more activity during the light period following the induction of the seizure paralleled the period of appetitive alterations that are similar to those displayed by animals with lateral hypothalamic lesions. That the activity of the suprachiasmatic hypothalamic nucleus was not significantly damaged (evident in Fig. 2) by the consequences of the lithium/pilocarpine-induced brain injury was indicated by the persistence of the diurnal rhythm (Reghunandanan, Reghunandanan, & Singh, 1993).

Although we did not measure melatonin levels directly, the vector (direction and magnitude) of the change in nocturnal activity but not in daytime activity is consistent with this assumption. Persistent nocturnal elevation of circulating melatonin, because of its anticonvulsant properties (Zeise & Semm, 1985), may have served as an ancillary inhibitor of ensembles of labile neuronal networks within the limbic system of these epileptic rats. Our observations (Michon & Persinger, 1997) that limbic epileptic rats exposed during the nocturnal cycle to specific shapes of magnetic fields that suppress nocturnal levels of melatonin (Reiter & Richardson, 1995; Yaga, Reiter, Manchester, Nieves, Sun, & Chen, 1993) exhibited increased proportions of overt seizures is consistent with this explanation.

Present results suggest that the agonistic behavior and male-male mounting (Persinger, 1994) exhibited by limbic epileptic rats are artifacts of the generalized increase in activity. If these animals were housed in groups, rather than singly (as in this study), the chance of confrontation would simply be increased; however, subsequent studies have shown that a significant com-

ponent of the aggression is temporally associated with increased geomagnetic activity (averaged over 24 hr.) over 40 nT (Persinger, 1997). This limen has been associated with the occurrence of more seizures during the same 2-hr. period as the contiguous geomagnetic activity or the application of experimental magnetic fields of similar intensities and whose shape simulated geomagnetic variations (Michon & Persinger, 1997). From this perspective the increased nocturnal activity would be derived from the same source of variance as the aggression and the seizures rather than being a nonspecific source whose modulation is responsible for the agonistic and paroxysmal behaviours. Nocturnal melatonin is considered a candidate for this source of variance.

A two-to-three-fold amplification of nocturnal activity would be consistent with disruptions in the inhibitory modulation of the processes that control the synthesis of melatonin or its circulating levels. If the persistence of the enhanced nocturnal activity were coupled to altered melatonin synthesis by the pineal gland, then a chronic alteration of normal afferents to the deep pineal region would be required. Cipolla-Neto, Bartol, Seraphim, Afeche, Scialfa, and Peracoli (1995) reported that exogenous electrolytic or chemical lesions within the intergeniculate leaflet of the thalamus, a region consistently damaged by the lithium/pilocarpine-induced seizures, altered the metabolic activity of the nocturnal pineal gland. Neurons within the leaflet contain a peptide (neuropeptide y) and a classical transmitter (GABA). Whereas the effect of the former appears to be variable (inhibitory or excitatory) depending upon subtle microstructural features, the latter is primarily inhibitory to the (nor)adrenergic stimulation of melatonin production. Consequently a selective, functional attenuation of the GABA component of these neurons would be expected to increase nocturnal pineal melatonin levels.

REFERENCES

Cipolla-Neto, J., Bartol, I., Seraphim, P. M., Afeche, S. C., Scialfa, J. H., & Peracoli, A. M. The effects of lesions on the thalamic intergeniculate leaflet on the pineal metabolism. *Brain Research*, 1995, 691, 133-141.

Michon, A. L., & Persinger, M. A. Experimental simulation of the effects of increased geomagnetic activity upon nocturnal seizures in epileptic rats. *Neuroscience Letters*, 1997, 224, 53-56.

Persinger, M. A. Maintained hypersexuality between male rats following chronically induced limbic seizures: implications for bisexuality in complex partial epileptic seizures. *Psychological Reports*, 1994, 74, 647-652.

Persinger, M. A. Geophysical variables and behavior: LXXXIII. Increased geomagnetic activity and group aggression in chronic epileptic male rats. *Perceptual and Motor Skills*, 1997, 85, 1376-1378.

Persinger, M. A., Bureau, Y., Kostakos, M., Peredery, O., & Falter, H. Behaviours of rats with insidious brain damage induced by seizures following single peripheral injections of lithium and pilocarpine. *Physiology and Behavior*, 1993, 53, 849-856.

Reghunandanan, V., Reghunandanan, R., & Singh, P. I. Neurotransmitters of the suprachiasmatic nucleus: role in the regulation of circadian rhythms. *Progress in Biology*, 1993, 41, 647-655.

REITER, R. J., & RICHARDSON, B. A. Magnetic field effects on pineal indoleamine metabolism and possible biological consequences *FASEB Journal*, 1995, 6, 2283-2287.

YAGA, K., REITER, R., MANCHESTER, L., NIEVES, H., SUN, J-H., & CHEN, L-D. Pineal sensitivity to pulsed magnetic fields during the photoperiod. *Brain Research Bulletin*, 1993, 30, 153-156.

ZEISE, M. L., & SEMM, P Melatonin lowers excitability of guinea pig hippocampal neurons in vitro. *Journal of Comparative Physiology*, 1985, 157, 23-29.

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